Table 9.2 Summary of nondissociable soluble aggregates of mAb1 in different formulations

	Soluble aggregates by SEC	Soluble aggregates by AUC	Viscosity (mPa s)
mAb1 reconstituted with SWFI[a]	0.2%	0.5%	60
mAb1 reconstituted with saline[b]	0.3%	0.6%	12
Lyophilized mAb1 stored at 60 °C, and reconstituted with SWFI[c]	11%	11%	41

SWFI, sterile water for injection.
[a]Lyophilized mAbl was reconstituted with 1.4 mL SWFI to final concentration of 125 mg/mL protein, 16 mM histidine, 266 mM sucrose, 0.03% polysorbate 20, at pH 6.0.
[b]Lyophilized mAbl was reconstituted with 0.9% saline to final concentration of 125 mg/mL protein, 16 mM histidine. 266 mM sucrose, 0.03% polysorbate 20, 128 mM NaCl, at pH 6.0.
[c]Lyophilized mAbl was stored at 60 °C for 1 week and then reconstituted with 1.4 mL SWFI to final concentration of 125 mg/mL protein, 16 mM histidine, 266 mM sucrose, 0.03% polysorbate 20, at pH 6.0.
From Liu et al. (2005).

high concentration. It can be expected that hydrophobic and electrostatic interactions are both present at high concentration and the domination of one versus the other would dictate formulation strategies to decrease viscosity. Thus, for some mAbs it has been shown that "hydrophobic" salts can disrupt interactions resulting in viscosity decrease (Du & Klibanov, 2011), whereas addition of ionic salts can decrease viscosity for other mAbs (Kanai et al., 2008; Liu et al., 2005). In the case of several mAbs it has been shown that the main attractive interactions are electrostatic in nature and that measured average dipole moments for mAb1 and mAb2 as a function of pH correlate with the DLS interaction parameter, k_D and G′ and viscosity measurements (Figure 9.10) (Singh, Yadav, Shire, & Kalonia, 2014). In particular, the magnitude of the mAb1 dipole moment increased from pH 4 to 6.5 and fell off sharply from pH 7 to 9.0, whereas the dipole moment for mAb2 with the same IgG_1 human Fc framework as mAb1 has its largest magnitude somewhere between pH 7 to 9, and is significantly lower at pH 6.5. Previously it was shown that the G′ storage modulus for mAb1was at a maximum at ~pH 6 at low ionic strength (Yadav, Shire, et al., 2012). The strength of the attractive interactions is reflected in the G′ value and this was corroborated by the negative values for k_D for mAb1 at pH 6 and low ionic strength (Yadav, Shire, et al., 2012). The measured dipole moments for mAb2 as a function of pH are also consistent with previously determined G′ and k_D values, which are maximum at pH 8. Finally, these observed pH trends for dipole moment, G′ and k_D for mAb1 and mAb2 correlate with the pH dependency of viscosity (Liu et al., 2005; Yadav, Shire, et al., 2012), suggesting that for mAb1 and mAb2, dipole interactions contribute significantly to the rheological properties of these mAbs at high concentration.

Computation of the electrostatic potential surfaces for mAb1 versus mAb2 showed that the Fab region of mAb1 had a substantially more negative potential surface than that of mAb2 (Figure 9.11) (Yadav, Laue, et al., 2012), which undoubtedly contributes to the presence of dipole–dipole interaction. The molecular basis for these differences will now be discussed.